Eluates from DAT-positive patients with or without hemolysis after high-dose IVIG yield predominantly IgG isoagglutinins of IgG_2 subclass

In an observational clinical study of IVIG-associated hemolysis, we previously showed that 32% to 36% of non–blood group O patients who receive IVIG will have some degree of hemolysis.[1,2] We also investigated whether the hemolysis can be explained by ABO zygosity, secretor status, or FcγR polymorphisms and found that only ABO zygosity is a significant risk factor, with group A_1B patients at greatest risk.[3] In a parallel study, we examined the IgG subclass of eluted IgG anti-A and/or anti-B from patients having a positive direct antiglobulin test (DAT) following IVIG administration of 2 g/kg, with or without hemolysis. The grading system used for hemolysis ranged from 0 (compensated) to 4 (severe) as previously described.[3] DATs were routinely performed using polyclonal rabbit anti-IgG and eluates were made using EluKit (Immucor) and stored at –80°C until they could be tested. A flow cytometric method was used to determine subclass, as previously described, with modifications.[4] The main modification of the flow method was that the eluates had to be concentrated (Ultracel-100 K cellulose centrifugal filters, Millipore) before testing (approximately 5- to 10-fold) to obtain enough antibody activity to perform the assay.

The patient cohort reported herein was a satellite study stemming from our larger prospective study on IVIG-associated hemolysis[1] and consisted of 22 patients, deliberately selected for subclass determination if they had a positive DAT following IVIG therapy. Of the 22 DAT-positive patients, 9 did not have evidence of hemolysis following IVIG therapy (DAT range, ± to 2+), while 13 showed evidence of hemolysis (DAT range, ± to 2+) using the Canadian IVIG Hemolysis Pharmacovigilance Group guidelines.[3,5] We were able to successfully determine the subclass of the IgG isoagglutinin in 10 patients. Two did not have evidence of hemolysis (Table 1). Of the 8 patients who showed evidence of hemolysis where the subclass of the eluted antibody could be determined, hemolysis ranged from mild/compensated to severe, Grade 4 (Table 1). IgG_2 isoagglutinins were predominant in all 10 patients, with 2 patients also having low amounts of IgG_1 (Table 1) and 3 patients having low levels of IgG_3 antibodies (Table 1). One group AB patient had IgG_3 anti-B (Table 1). Despite concentrating the eluates, 8 failed to give a positive result in our flow assay and for 3, although testing of the eluate using flow against A or B RBCs was positive, there was not enough sample remaining for subclass testing. Failure to detect antibody in the eluates may have been due to low amounts of antibody eluted from the RBCs as well as long-term storage causing degradation of the antibody, as eluates prior to testing for subclass had been stored up to 2 years; and, although stored at –80°C, there was no added protein or another agent to help stabilize the antibodies.

IgG subclasses possess different characteristics in terms of binding to their different receptors in immune cells and activation of complement. Whereas human IgG_1 and IgG_3 activate the classical complement cascade similarly, IgG_2 does it poorly and IgG_4 does not activate complement.[6] Complement activation has not been reported following

TABLE 1. Results of IgG subclass testing of eluates from patients who developed a positive DAT following IVIG therapy

Patients characteristics*				Total IgG bound to RBC		IgG_1 bound to RBC		IgG_2 bound to RBC		IgG_3 bound to RBC		IgG_4 bound to RBC		Hemolysis/ Grade[3,5]	Comments
ID	Sex	ABO	DAT	A	B	A	B	A	B	A	B	A	B		
1	F	AB	±	+++	–	+	ND	+++	ND	–	ND	–	ND	Yes/1	
2	F	A	±	+++	–	–	ND	++	ND	+	ND	–	ND	Yes/compensated	
3	F	AB	±	+++	+	–	–	++	–	+	±	–	–	Yes/2	
4	F	A	±	+++	–	–	ND	+	ND	±	ND	–	ND	No	
5	F	AB	+	++	–	–	ND	++	ND	–	ND	–	ND	Yes/4	
6	M	A	++	++	–	–	ND	++	ND	–	ND	–	ND	Yes/4	
7	M	A	++	+	–	±	ND	++	ND	–	ND	–	ND	Yes/3	
8	F	A	±	+	–	–	ND	+	ND	–	ND	–	ND	Yes/3	
9	M	A	+	+	–	–	ND	+	ND	–	ND	–	ND	Yes/4	
10	M	A	±	+	–	–	ND	±	ND	–	ND	–	ND	No	Received 1 g/kg IVIg

* Patients organized based on the strength of the fluorescence-activated cell sorting results for total IgG followed by the intensity of the subclass testing. +++, strongly positive; ++, very positive; +, positive; ±, weakly positive; –, negative for flow cytometry analysis. DAT = direct antiglobulin test; ND = not determined (unnecessary or because of insufficient material).

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IVIG-associated hemolysis[2]; therefore, only IgG was tested by DAT. As the ABO system is a carbohydrate antigen system,[7] it was not surprising that the isoagglutinins eluted from the DAT-positive patient samples were of the IgG_2 subclass.[4,6] This subclass is known to be associated with carbohydrate antigens and studies previously reported have shown that ABO isoagglutinins are primarily of the IgG_2 subclass.[4] IgG_2 subclass antibodies also are known to interact with the FcγRIIA receptor on mononuclear phagocytes,[3,6,8,9] and this interaction can be enhanced by specific polymorphisms.[8,9] Although the patients reported herein were not tested for FcγRIIA polymorphisms, we previously showed in a different cohort of patients with or without hemolysis that there is no correlation of FcγR polymorphisms with IVIG-associated hemolysis.[3]

Despite the caveat that only 10 of 22 patients' DAT-positive subclass could be determined, these results can still provide some valuable conclusions, as we were able to test 8 patients who hemolyzed and 2 patients who did not hemolyze. We conclude that in patients who develop a positive DAT following IVIG therapy the predominant antibody opsonizing the patients' autologous RBCs is an isoagglutinin of IgG_2 subclass. We further conclude that there is no correlation of IgG_2 or other subclasses to whether a patient will hemolyze or not.

CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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ABO-associated antibody-mediated rejection following A2B-to-B renal transplantation and successful treatment with therapeutic plasma exchange

Organ allocation policies allowing blood type A2(B) donor to B recipient renal transplantation came into effect in December 2014 as an effort to increase allograft access for blood type B transplant candidates. For B candidates to be listed to receive A2(B) donor kidneys, a pretransplantation anti-A IgG titer using A2 cells (A2-IgG) must be 4 or less ("low"). Long-term follow-up has demonstrated that A2(B)-to-B transplants with previously low A2-IgG titers have noninferior clinical outcomes when compared to B-to-B transplants.[1,2]

We report a case of a 64-year old B-positive male with end-stage renal disease secondary to type 2 diabetes mellitus, cirrhosis, and hypertension, who had been actively listed for a renal transplant for 2 years. At the time of his transplant from an A2B donor, his panel reactive antibodies demonstrated 0% reactivity for both HLA class I and class II antigens, and his A2-IgG was 1:1 or 1:2 by doubling dilution tube method using the patient's serum and A2 cells on four occasions; the closest testing was 52 days before the transplant. Per institutional protocol, his immunosuppression regimen consisted of

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